RNA Applications

RNA QA/QC
Microarrays

Gene
Expression

RNA QA/QC
qPCR

RNA QA/QC
mPCR

smallRNA
QA/QC

Intact RNA: RIN 10

Partially degraded RNA: RIN 5

Strongly Degraded RNA: RIN 3
RNA QC Workflow
Analysis of Total RNA Integrity

Cells / Culture
RNA isolation
New or Improved sample isolation

RNA QC via 2100 Bioanalyzer
RNA Integrity Number (RIN) as independent and standardized measure of sample quality

RIN

RIN above threshold

Continue with downstream Experiment (Microarray, real-time PCR, etc.)

Now - even for Plant RNA!

Typical first QC step after RNA sample prep prior to microarrays or real-time PCR

High quality total RNA
RIN 9.2

Partially degraded total RNA
RIN 5.8

2100 bioanalyzer: electrophogram

Agilent Technologies
RIN Application – Assessment of RNA Integrity

Intact RNA: RIN 10

Partially degraded RNA: RIN 5

Strongly Degraded RNA: RIN 3
Workflow: QPCR with Bioanalyzer QC

1. RNA extraction
   - Stratagene Absolutely RNA® MiniPrep kit

2. RNA degradation
   - RNA 6000 Nano kit

3. RNA QC and quantification
   - Stratagene AffinityScript™
   - Agilent 2100 bioanalyzer

4. Reverse transcription
   - DNA 1000 kit

5. QPCR
6. QPCR assay validation
RNA QC/qPCR Sales Tools

- RNA integrity/qPCR CD 5989-9911EN - Distributed more than 3000 CDs worldwide
  - Publications, References
  - Application Notes
  - Product Information
  - Weblinks

- RNA QC/qPCR Application Note (5989-7730EN)

- Customer Presentation

- External References and publications
External References – qPCR News (e-Newsletter)

qPCR e-Newsletter May Issue:
Focus on RNA quality control
Links to list of RNA integrity publications
MIQE: Minimum Information for publication of qPCR experiments

Almost all listed publications refer to the Bioanalyzer for RNA QC
Laser Microdissection – PALM MicroBeam System and RNA Pico kit

Laser microdissection

Laser pressure catapulting: Section after catapulting of selected area

Catapulted area in the collection device

Laser Microdissection and Pressure Catapulting (LMPC)

RNA extraction

RNA sample QC using the Agilent 2100 bioanalyzer and the RNA 6000 Pico LabChip kit

Data kindly provided by P.A.L.M. Microlaser Technologies
New Small RNA Assay versus existing RNA Assay

**RNA 6000Nano**
- Size range: 25-6000nt
- Results: Integrity, Total RNA amount, gDNA contamination

- **RNA 6000Nano kit**
- **Small RNA Kit**

**NEW! Small RNA**
- Size range: 6-150nt
- Results: miRNA amount, Ratio and amount of other Small RNA
DNA Applications

mPCR analysis
Gene Expression
Restriction Digest
Food Analysis
Forensic Testing

Graphs showing DNA analysis results.
Determination of PCR Product Impurity

Quantitative data from Agilent 2100 bioanalyzer

Sample               c (DNA) main peak
300 bp PCR           41.4 ng/ul  40.7 ng/ul
300 bp PCR 1:4       9.6 ng/ul   9.6 ng/ul

Impurity level : < 2%

Impurity level : > 50%

Quantitative data from Agilent 2100 bioanalyzer

Sample               c (DNA) main peak
3000 bp PCR          61.9 ng/ul  40.7 ng/ul
3000 bp PCR 1:4      14.8 ng/ul  9.8 ng/ul
Optimization of Multiplex PCR on a 19-plex PCR

Data kindly provided by QIAGEN GmbH, Germany
Next Generation Sequencing is an emerging technology.

Quality of sequences is one of the chief technical concerns about next generation sequencing platforms in a recent customer survey by Insight pharma reports.

Leading next generation sequencing vendors (Illumina, Roche, Life Technologies) recommend existing Bioanalyzer DNA assays in their workflows.

- Bioanalyzer DNA kit: ~30% growth in FY08
- Growth potential: still NGS users who just use slab gel and Nanodrop
Next Generation Sequencing Workflows
Bioanalyzer Quality Control officially recommended by: Roche - Illumina - ABI

Recommendation: use of RNA and DNA solutions by Agilent 2100 Bioanalyzer

Key Scientific Reference:

Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SA, UK. Correspondence should be addressed to D.J.T. (dit@sanger.ac.uk).

PUBLISHED ONLINE 25 NOVEMBER 2008; DOI:10.1038/NMETH.1270

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DNA QC in Next-Gen Sequencing – Illumina DNA Library Prep Workflow

Simplified Illumina GAII Workflow

Illumina Sequencing

Starting Material: Genomic DNA

Fragment DNA

Ligate Adapters

Purify Ligation Products

Amplify & Quantify Library

Sequence Library

Agilent 2100 Bioanalyzer

Check Size Distribution

Quantify, Size and QC DNA libraries
New High Sensitivity DNA Kit - Product Structure

- Bioanalyzer kit for sizing and quantification of dsDNA fragments and dsDNA smears in the 50 – 7000 bp size range down to pg/µl sensitivity
- Product structure follows standard DNA kit structures
- 10 Chip Kits (11 samples/chip)
- SW upgrade to 2100 expert B.02.07 required

Agilent High Sensitivity DNA Kit (5067-4626)
High Sensitivity DNA Reagents (5067-4627)
Protein Applications

Protein Purification
Protein Expression
Protein Production
Food Analysis
Purity and QA/QC

![Image of Protein Chip]

**ng protein loaded**

- Silver stained PA-gel
- 2100 Pseudo gel view

**Graph:**

- Signal to Noise
- Concentration of labeled BSA on-chip in pg/μL

\[ y = 12.791x^{0.8576} \]
\[ R^2 = 0.9964 \]
Quality Control of Antibodies

Determine the half antibody content in IgG preparations

Antibody analysis under reducing and non-reducing conditions

Absolute Quantitation of IgG samples

16% half antibody

90 kDa 160 kDa

light + heavy chain

intact antibody

light chain

heavy chain

Ab reduced

Ab non-reduced
Bioanalyzer: Protein Kit portfolio

- Standard Kits (P80,P230) for fastest analysis in average concentration range
- High Sensitivity Kit (HSP-250) for pg amounts and extra wide linear quantification ranges

- **Protein 80 kit**
  Prod No 5067-1515
  Sizing: 5 – 80 kDa
  Protein staining on Chip
  Sensitivity: Coomassie (ng/µL)
  25 Chip Kit (Max 250 samples)
  Access to Small Proteins
  Quick sample prep
  Easy to use

- **Protein 230 kit**
  Prod No 5067-1517
  Sizing: 14 – 230 kDa
  Protein staining on Chip
  Sensitivity: Coomassie (ng/µL)
  25 Chip Kit (Max 250 samples)
  Standard protein range
  Quick sample prep
  Easy to use

- **HSPProtein 250 kit**
  Prod No 5067-1575
  Sizing: 10 – 250 kDa
  Covalent labeled Proteins
  Sensitivity: Silver (pg/µL)
  10 Chip Kit (Max 100 samples)
  Highest Sensitivity
  4 Orders linear range
  Labeling reaction included

- Antibodies
- Protein QA/QC
- Abundant Proteins
- Expression Optimization
- Recombinant Proteins

- Low abundant Proteins
- Impurity analysis
- Antibodies
- Protein QA/QC
Cell Applications

Protein Expression  Apoptosis Detection  Gene Silencing  Transfection Monitoring  Cell Staining Inside / outside

Det plot view for easy data evaluation

Video